

Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα

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The initiation of immunoglobulin-E (IgE)-mediated allergic responses requires the binding of IgE antibody to its high-affinity receptor, FcεRI. Crosslinking of FcεRI initiates an intracellular signal transduction cascade that triggers the release of mediators of the allergic response. The interaction of the crystallizable fragment (Fc) of IgE (IgE-Fc) with FcεRI is a key recognition event of this process and involves the extracellular domains of the FcεRI α-chain. To understand the structural basis for this interaction, we have solved the crystal structure of the human IgE-Fc–FcεRIα complex to 3.5-Å resolution. The crystal structure reveals that one receptor binds one dimeric IgE-Fc molecule asymmetrically through interactions at two sites, each involving one Cε3 domain of the IgE-Fc. The interaction of one receptor with the IgE-Fc blocks the binding of a second receptor, and features of this interaction are conserved in other members of the Fc receptor family. The structure suggests new approaches to inhibiting the binding of IgE to FcεRI for the treatment of allergy and asthma.

The high-affinity IgE receptor (FcεRI) is found on the surface of effector cells of the immune system that initiate cellular reactions associated with the allergic response, anaphylaxis and anti-parasitic immunity^{1,2}. The human receptor can form either a trimeric αγ₂ or tetrameric αβγ₂ structure on cell surfaces, with the extracellular domains of the α-chain conferring the ability to bind antibodies of the IgE class with high affinity ($K_d \approx 10^{-9}$ – 10^{-10} M). IgE antibodies are heterotetramers of two heavy and two light chains (H₂L₂), with the FcεRI-binding domains located in the carboxy-terminal Fc region of the heavy chain. The dimeric Fc fragment of the IgE antibody has full binding activity for FcεRI (refs 3–5). IgE antibodies bind to the receptor in the absence of antigen, and thus the receptor adopts the antigenic specificity of the prevalent IgE repertoire. Crosslinking of the receptor through antigen/antibody interactions leads to the initiation of a signal transduction cascade mediated by lyn and syk kinases, analogous to that induced by T-cell and B-cell receptors^{6–8}. In mast cells, receptor activation leads to rapid degranulation and the release of histamine, followed by the synthesis and release of prostaglandins, leukotrienes, cytokines and other mediators of the allergic response. Anti-parasitic responses can be triggered through a similar activation of eosinophils, leading to the release of granular proteins toxic to schistosomes and other parasites. FcεRI belongs to a family of antibody-binding receptors that also mediate interactions of soluble IgG antibodies with cells of the immune system^{6,8}.

Atopic diseases such as allergy, asthma and eczema comprise a wide spectrum of pathologies associated with the inappropriate activation of the immune system by environmental antigens⁹. Marked increases in atopic disease have been observed in the past century, particularly in developed countries. Genetic studies in both mice and humans have shown the association of the IgE network with allergic diseases, suggesting that polymorphisms of the FcεRI β-chain and CD14 are involved in atopic individuals⁹. The interaction of the IgE antibody with FcεRI is central to these immune reactions, and provides an attractive target for the inhibition of all IgE-mediated allergic disease. Clinical studies of allergic individuals using anti-IgE monoclonal antibody therapy has shown that this is an effective approach to disease treatment^{10,11}.

We have reported the crystal structure of the α-chain ectodomains of human FcεRI (ref. 12), which revealed a highly bent arrangement of two immunoglobulin domains. Four solvent-exposed tryptophans cluster at the top of the receptor, forming a large hydrophobic surface for potential interactions with the IgE-Fc. This tryptophan cluster borders the Fc-binding site that was previously mapped by mutagenesis studies, which implicated residues in the second domain of the receptor in IgE binding. The structural and functional data suggested that a large convex surface of the receptor could be involved in binding IgE, raising questions about the role of the tryptophans, the convex nature of the binding site, and the mechanisms underlying the stoichiometry and binding specificity for IgE. Given the dimeric structure of the IgE-Fc, which could potentially display two receptor binding sites, the structural basis for the observed stoichiometry of one IgE binding to one receptor has remained obscure.

To address these questions, we have solved the crystal structure of a complex of the human IgE-Fc bound to FcεRIα. The structure of the complex reveals two interaction sites for the IgE-Fc on the receptor surface and clarifies how a 1:1 complex between antibody and receptor is formed. The two Cε3 domains of IgE-Fc bind to distinct sites on the receptor: one is formed by the C–C' loop in the receptor D2 domain; the second involves the four solvent-exposed tryptophans. The structure of the complex accounts for previous mutagenesis and structural observations and shows that the Fc forms a complementary arch across the convex surface of the receptor. The IgE-Fc–FcεRIα complex provides a model for understanding the function of other antibody Fc receptors, and suggests new approaches to the inhibition of diseases mediated by IgE.

Structure determination of the complex

We expressed IgE-Fc and FcεRIα in insect cells using recombinant baculovirus technology. The expression of FcεRIα was carried out as described¹². The IgE heavy chain contains four constant domains (Cε1–Cε4), in contrast to the three found in IgG antibodies. The interaction of FcεRI with IgE has been mapped to the two C-terminal constant domains of the IgE-Fc (domains Cε3–Cε4)^{3,13–16}. Deglycosylated IgE-Fc retains comparable affinity for FcεRIα, but

aggregates into a less active form³. The treatment of cells that express IgE with oligosaccharide-processing inhibitors that limit glycosylation to a high-mannose form revealed no effects on FcεRI binding¹⁷.

The Cε3–Cε4 domains of human IgE–Fc isolated from insect cells are modified with high-mannose carbohydrate at the conserved glycosylation site at N394 (B.A.W. *et al.*, manuscript in preparation), and form stable complexes with FcεRIα. Using wild-type FcεRIα protein, crystals of the complex grow in space group *P*₄₁₂₁₂; however, diffraction data are limited to a resolution of about 4.5 Å (Table 1, crystal form I). To improve the crystals, we expressed a triple carbohydrate mutant of FcεRIα (FcεRIαΔ4–6) in insect cells. The FcεRIαΔ4–6 mutant lacks carbohydrate attachment sites at three of the seven wild-type locations (residues 74, 135 and 140)¹⁸. Complexes formed with FcεRIαΔ4–6 expressed in baculovirus grow crystals in space group *R*32 and diffract X-rays to a resolution of 3.5 Å (Table 1, crystal form II). The structure was determined by molecular replacement techniques (see Methods). Current refinement statistics for the complex are shown in Table 1, with an overall *R*_{free} of 27.3% and *R*_{work} of 25.4% to 3.5 Å. Figure 1a illustrates electron density from a σ_a -weighted $2F_o - F_c$ simulated annealing omit map calculated with the current model phases. Both crystal forms of the IgE–Fc–FcεRIα complex show a 1:1 complex with similar overall features. Interpretation of the structure is limited to crystal form II because of the low resolution of crystal form I.

Overview of the complex

The receptor contains two distinct binding sites for the antibody (Fig. 1b–d). Binding interactions are formed exclusively between the Cε3 domains of the IgE–Fc and FcεRIα. The Cε3–Cε4 domain interfaces and the Cε4 domains do not make receptor contacts. The two Cε3 domains are related by a nearly perfect dyad axis (180.7° rotation), except for residues in the Cε2–Cε3 linker region (residues 328–336). The Cε4 domains are also related by a nearly perfect dyad axis (179.6° rotation), which is offset 3° from the Cε3 pseudo-dyad (see below). Electron density for the terminal residues of each chain is weak, including the amino-terminal interchain disulphide of the IgE–Fc.

There are *N*-linked oligosaccharides in both the IgE–Fc and FcεRIα (Fig. 1b–d), but they do not contribute significantly to interactions between the two molecules. Carbohydrate attached to the conserved site (N394 in IgE), lies along the inner face of the Cε3

domains (strands A, B and E) similar to that observed in structures of IgG–Fc. Carbohydrate attached to residue N42 in FcεRIα extends upwards to the top of the D1–D2 interface approaching one of the IgE–Fc chains (Fig. 1b–d). Carbohydrate residues are attached to N21 and N166 of FcεRIα, projecting from the receptor surface away from the IgE-binding interface. We did not model carbohydrate for N50 of FcεRIα, nor N371 or N383 of IgE.

The Cε3 domains of the heavy chain dimer of IgE bind along one side of the D2 domain and at the top of the D1–D2 interface of FcεRIα, defining two distinct interaction sites. Site 1 refers to the interaction of one Cε3 domain with the C–C' region of the receptor D2 domain, centred around receptor residue Y131. Site 2 refers to the interaction of the second Cε3 domain with the D1–D2 interface (Fig. 1b, c), and involves the cluster of four surface-exposed tryptophans (W87, W110, W113 and W156). The two chains of the IgE–Fc bind the receptor using surface loops in Cε3 (Fig. 2a), including the immunoglobulin-fold of BC loop (362–365), DE loop (393–396), FG loop (424–427), and the Cε2–Cε3 linker region (332–336). Interactions with FcεRIα cause the Cε2–Cε3 linker regions to point up and away from the complex interface. The Cε4 domains of the IgE–Fc provide a structural dimerization scaffold that allows two Cε3 domains to form the bivalent interaction with FcεRIα.

Structural basis for the formation of a 1:1 complex

Biophysical studies of the IgE–Fc–FcεRIα complex indicate that a 1:1 complex is formed between the antibody and receptor^{4,5,19,20}, consistent with the crystallographic structure. This contrasts with models proposing a 1:2 stoichiometry for the homologous IgG–Fc receptors FcγRIIa²¹ and FcγRIIb^{22,23}. Fc receptors belonging to other structural classes can form complexes with a 1:2 stoichiometry, such as the major histocompatibility complex (MHC)-like neonatal Fc receptor²⁴, or use two identical domains to bind one antibody, such as the lectin-like low-affinity IgE receptor FcεRII (ref. 25). The 1:1 stoichiometry of the IgE–Fc–FcεRIα complex ensures that receptor crosslinking and cellular activation depend upon multivalent antigen binding.

Figures 1b–d and 2 show surface and ribbon representations of the IgE–Fc–FcεRIα complex. The two Cε3 domains of IgE interact with FcεRIα using surface loops that are structurally analogous to the complementarity determining region loops of antibody variable

Table 1 Data collection and refinement statistics

Data	Form I	Form II, low resolution	Form II, high resolution
Data Set			
Resolution (Å) *	30–4.50 (4.66–4.50)	30–4.00 (4.14–4.00)	40–3.5 (3.63–3.5)
Source	APS DND 5ID	ALS 5.0.2	APS DND 5ID
Wavelength (Å)	1.000	1.200	1.034
Completeness*	99.5 (97.7)	99.7 (98.3)	99.8 (99.7)
Average redundancy*	7.0 (5.8)	5.0 (3.9)	3.1 (3.1)
<i>R</i> _{sym} (%)†	17.8 (57.5)	15.2 (75.0)	9.1 (46.1)
$\langle I/\sigma \rangle$ *	5.9 (2.0)	4.4 (2.0)	16.5 (2.7)
No. of observed / no. of unique reflections	39,925 / 5,703	91,617 / 18,459	84,873 / 27,484
No. of working / no. of free *		18,455 / 945	25,993 / 1,418 (2,543 / 159)
<i>R</i> _{work} / <i>R</i> _{free} (%)‡			25.4 / 27.3 (31.6 / 37.2)
Refinement (form II, 3.5 Å)			
No. of atoms	Total	Protein	Carbohydrate
	5,251	4,821	259
		Detergent	Sulphate
		146	25
		Water	0
R.m.s. Deviations	Bonds	Angles	Dihedrals
	0.0100 Å	1.55°	25.4°
			Improper
			1.23°
Average <i>B</i> (Å ²)	Overall	Receptor	Fc chain 1
	88.0	68.1	97.8
			Fc chain 2
			99.2
Ramachandran	Favoured	Allowed	Generous
	77.0%	21.5%	1.5%
			Disallowed
			0.0%

* Last shell is shown in parentheses.

† $R_{\text{sym}} = \sum_i \sum_j |I_{ij} - \langle I_i \rangle| / \sum_i \sum_j I_{ij}$, where I_{ij} is the i^{th} intensity measurement of reflection h and $\langle I_i \rangle$ is the average intensity of that reflection.

‡ $R_{\text{work}}/R_{\text{free}} = \sum_h |F_o - F_c| / \sum_h |F_o|$, where F_c is the calculated and F_o is the observed structure factor amplitude of reflection h for the working/free set, respectively.

domains (Fig. 2a). The two distinct binding surfaces of the receptor (Fig. 2b) establish a convex and asymmetric interaction with the two $\text{Ce}3$ domains, placing the receptor near the $\text{Ce}3$ pseudo-dyad axis. There are two structural keys that dictate the formation of complexes with 1:1 stoichiometry: first, induction of structural asymmetry in the $\text{Ce}2$ – $\text{Ce}3$ linker of IgE-Fc; and second, steric hindrance that blocks the binding of a second receptor.

Superimposing the two $\text{Ce}3$ domains in the IgE-Fc structure (Fig. 2c) shows that the $\text{Ce}2$ – $\text{Ce}3$ linker regions (residues 328–336) are constrained to an asymmetric arrangement by interactions with $\text{Fc}\epsilon\text{RI}\alpha$. The $\text{Ce}4$ domains are not optimally aligned by the superposition of the $\text{Ce}3$ domains, owing to the 3° difference in the $\text{Ce}3$ and $\text{Ce}4$ pseudo-dyad axes. Independent movement of $\text{Ce}3$ and $\text{Ce}4$ domains within one heavy chain might allow optimal binding interactions to form in the complex. The BC and FG loops also adopt slightly different conformations in the two $\text{Ce}3$ domains because of their interactions with the two binding sites of $\text{Fc}\epsilon\text{RI}\alpha$.

Binding of one receptor to the IgE-Fc creates a steric block to binding a second receptor. Figure 2d–f shows representations of both the IgE-Fc and $\text{Fc}\epsilon\text{RI}\alpha$ in which the complex has been separated to expose the buried interaction surfaces. The amino acids of the $\text{Ce}2$ – $\text{Ce}3$ linker form the top of an arch that conforms to the convex surface of $\text{Fc}\epsilon\text{RI}\alpha$, creating an asymmetric binding site for a single receptor. In addition, four IgE residues (R334, G335, V336 and H424) are common to both site 1 and site 2, preventing the simultaneous binding of two receptors to one IgE-Fc. Superposition of a second receptor onto the 1:1 complex shows significant steric overlap between receptors and the amino acids of the $\text{Ce}2$ – $\text{Ce}3$ linker. Thus, the binding of one receptor effectively prevents the binding of a second, owing to both the induced asymmetry of the $\text{Ce}2$ – $\text{Ce}3$ linker of the IgE-Fc and the steric inhibition across the Fc dyad axis.

Structural changes in the receptor and IgE on binding

The receptor shows little change in conformation on complex formation with the Fc. The overall r.m.s. difference in 158 $\text{C}\alpha$ positions is 1.11 Å compared with the unbound receptor, with little change in the angle between D1 and D2 (ref. 12). Two loops on the receptor adopt conformations different from those in the uncomplexed $\text{Fc}\epsilon\text{RI}\alpha$ structure, the C' strand in D2 (residues 127–133) and the BC loop in D1 (residues 30–35). The C' strand of D2 is longer in the complex; residues L127–Y131 form additional hydrogen bonds to the C strand. Analysis of the $\text{Fc}\epsilon\text{RI}\alpha$ structure in several crystal forms (S. C. G. *et al.*, manuscript in preparation), however, shows that the C' strand can adopt this conformation in the absence of the IgE-Fc. The BC loop in D1 also adopts different conformations in different crystal forms, but this region is distal to the IgE-Fc binding site.

In the complex, the IgE-Fc is in a conformation that is similar to the Fc domains of IgG antibodies^{26,27}, but the structure of the uncomplexed IgE-Fc reveals structural differences that could influence receptor binding (B. A. W. *et al.*, manuscript in preparation).

Binding surfaces of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ interaction

Formation of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ complex buries a binding surface totalling about 1,850 Å² (Fig. 2d–f). Site 1 and site 2 use overlapping but non-identical sets of IgE residues. Of the fifteen $\text{Fc}\epsilon\text{RI}\alpha$ residues within 4 Å of the IgE-Fc, seven are aromatic—of which five are surface-exposed tryptophans. In contrast, of the eighteen IgE-Fc residues within 4 Å of $\text{Fc}\epsilon\text{RI}\alpha$, none are aromatic. The large number of aromatic receptor residues and the large buried surface area may contribute to the stability of the complex ($K_d \approx 10^{-9}$ – 10^{-10} M).

Figure 3a shows a plot of the IgE-Fc residues that are buried by interaction with the receptor. Residues of the $\text{Ce}3$ domain located in site 1 (top half of Fig. 3a) form specific interactions with the residues

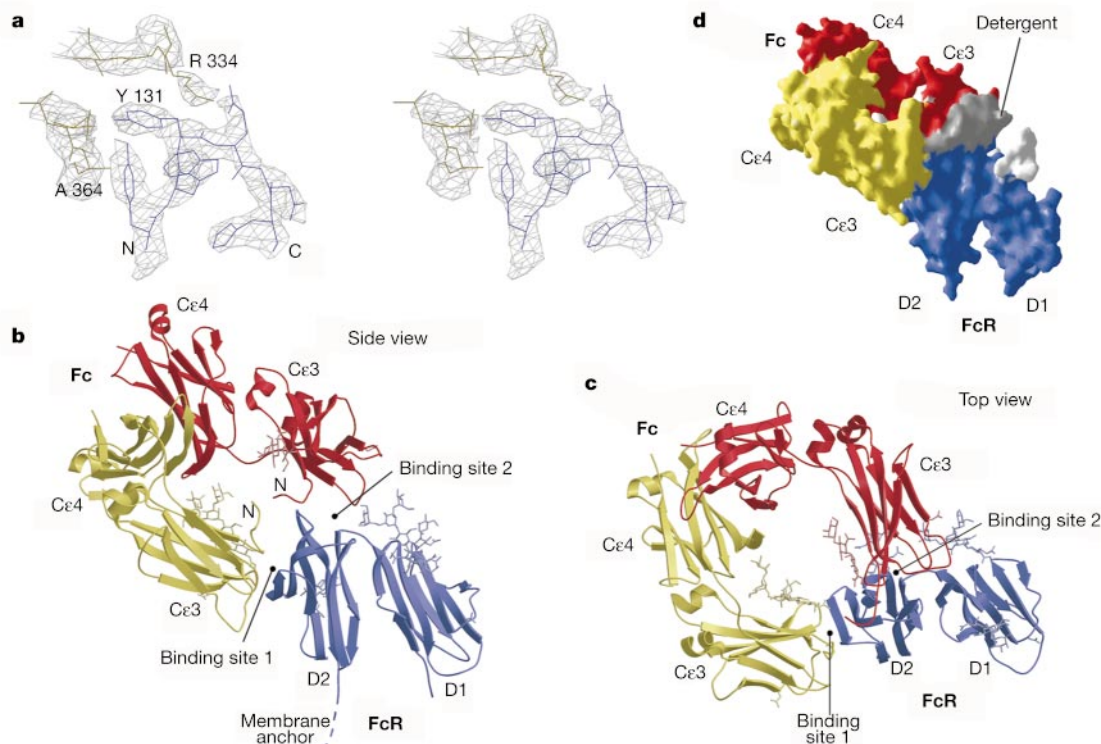


Figure 1 Overview of the crystal structure of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ complex. **a**, Example electron density for binding site 1. Shown is a stereo diagram from a σ_A -weighted $2F_o - F_c$ simulated annealing omit electron-density map at 3.5 Å calculated using the final refined structure of the complex contoured at 1.25 σ . Residues 129–136 of $\text{Fc}\epsilon\text{RI}\alpha$, and loop residues 334–336 and 362–364 of IgE-Fc are shown. **b**, Side view of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ complex. The two Fc chains are in yellow and red, the $\text{Fc}\epsilon\text{RI}\alpha$ chain is in blue.

Carbohydrate residues are shown as sticks in the same colour as the protein chain to which they are attached. The colour scheme is the same in all figures. Binding sites 1 and 2 are indicated. The cell membrane would lie below the receptor. **c**, Top view of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ complex. **d**, Side view of a surface representation of the IgE-Fc– $\text{Fc}\epsilon\text{RI}\alpha$ complex highlighting the asymmetric interactions on the receptor surface. Carbohydrate surfaces are white, detergent surface is grey.

of FcεRIα shown in Fig. 3b. Twelve amino acids from the IgE and eight amino acids from the receptor form site 1, burying a total of about 860 Å² of surface area. The IgE residues are from four distinct regions of the IgE-Fc sequence, including the Cε2–Cε3 linker (residues 334–336), the BC loop (362–365), the DE loop (393–396) and the FG loop (residue 424). The first *N*-acetylglucosamine attached at the conserved N394 site buries 50 Å² in site 1. N394 and the attached carbohydrate are not involved in site 2. The limited involvement of the carbohydrate is consistent with biochemical studies showing that it is not required for receptor binding³. The receptor residues derive from two regions of the D2 domain, involving the C strand (residues 117, 119 and 121) and the C'–E region (residues 126 and 129–132). Two potential salt bridges (αK117–Cε3D362 and αE132–Cε3R334) and four potential hydrogen bonds (αK117–Cε3G335, αY129–Cε3D362, αY131–Cε3D364 and αY131–Cε3H424) are formed across the site 1 interface (Fig. 3b). Y131 from the receptor projects into a pocket

on the IgE-Fc formed by the BC and FG loops of Cε3 and the Cε2–Cε3 linker (Figs 2d–f and 3b).

The residues the Cε3 domain that are buried in the formation of site 2 are shown in Fig. 3a and c. Interactions at site 2 bury about 970 Å², including ten amino acids from IgE and eight from FcεRIα. The IgE residues are localized to two distinct segments of Cε3: the Cε2–Cε3 linker region (residues 332–337); and the FG loop (424–427). The FcεRIα contributes residues from three regions: the D1–D2 linker region (85–87); the D2 BC loop (residues 110 and 113); and the D2 FG loop (156–158). Residues from the D1 domain of the receptor do not form direct interactions with the IgE-Fc. Site 2 primarily contains hydrophobic amino acids with three potential hydrogen bonds across the interface (αW156–Cε3G335, αQ157–Cε3N332 and αQ157–Cε3R334). P426 from the IgE-Fc intercalates between receptor residues W87 and W110, forming a hydrophobic proline sandwich (Figs 2d, e and 3c). These three residues are conserved in IgG antibodies and the IgG receptor family.

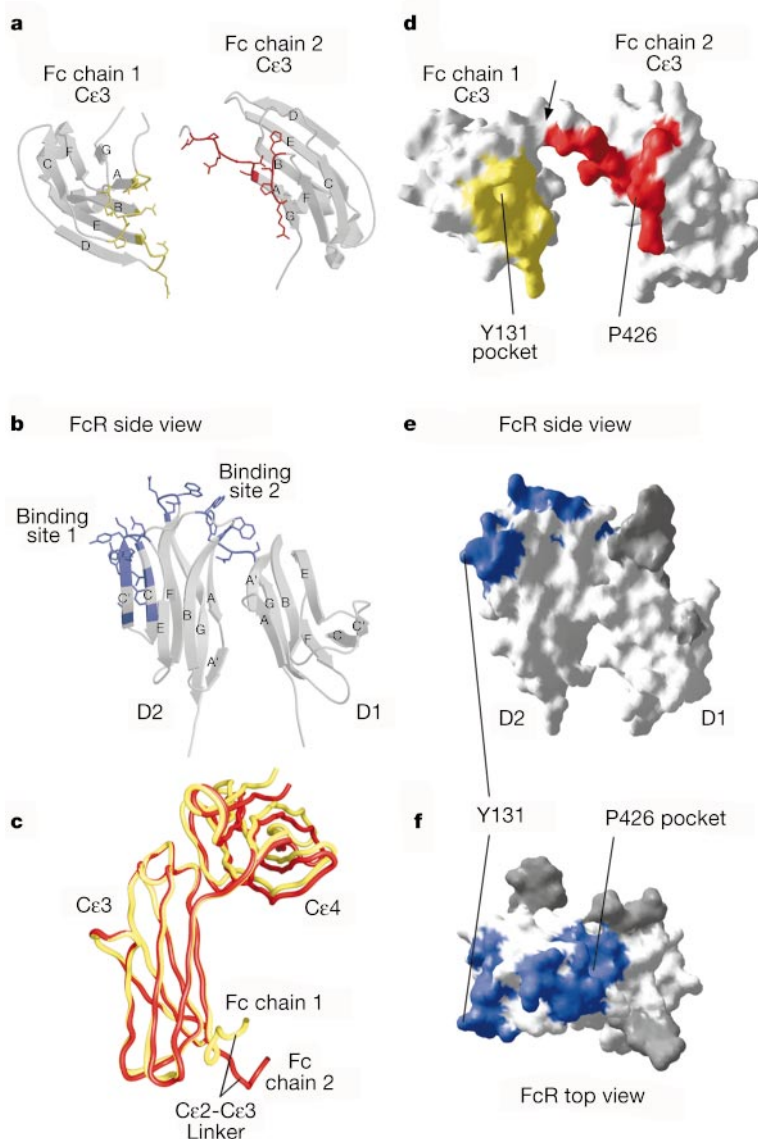


Figure 2 Structural basis for the 1:1 binding of IgE to FcεRIα. The IgE-Fc–FcεRIα complex has been taken apart to display the interaction sites. **a**, End-on view of the Cε3 domains of IgE. Immunoglobulin domain strands are labelled, and binding residues that bind FcεRIα are shown as sticks in yellow (site 1) and red (site 2). **b**, Side view of FcεRIα. Strands in D1 and D2 are labelled, and residues that bind IgE are shown as sticks on the ribbon surface. **c**, Superposition of the two Cε3 domains of IgE-Fc. The twofold symmetry of the IgE-Fc domains is broken in the Cε2–Cε3 linker region (328–336) by interactions

with the receptor. Superposition of the Cε3 domains leads to a small displacement in the Cε4 domains, because of a 3° difference in Cε3 and Cε4 pseudo-dyad axes. **d**, Surface view of the Cε3 domains of IgE in the same orientation as in **a**. The surface buried by FcεRIα is coloured yellow (site 1) or red (site 2). The binding pocket for receptor residue Y131 is shown, as is the conserved P426 on the Fc. An arrow indicates the Cε2–Cε3 linker. **e**, **f**, FcεRIα residues buried upon formation of the complex. Residue Y131 of site 1 and the binding pocket for P426 of the IgE-Fc are labelled. Carbohydrate is shown in grey.

Electron density is observed for CHAPS detergent in the form II crystals, with one molecule located above W156 of FcεRIα and below the FG loop of Ce3 near H424 in site 2 (Fig. 3d). Although the CHAPS interaction may be weak, this structure provides a foundation for using combinatorial synthetic chemistry methods to improve these initial binding interactions²⁸. The position of the CHAPS steroid core is analogous to the position of Y131 of FcεRIα in site 1, and adding atoms to the CHAPS scaffold could disrupt the interactions in site 2. H424 of the IgE-Fc, which is located next to the CHAPS binding site, makes contacts with the receptor in both site 1 and site 2. A small molecule inhibitor that could interact with both the Y131 pocket of Ce3 (site 1) and with H424 might effectively disrupt both site 1 and site 2 interactions with the receptor.

Locations of IgE and FcεRIα mutations

Mutagenesis studies of both the IgE-Fc and FcεRIα have been carried out in efforts to define the residues contributing to the stability of the complex. For FcεRIα, these studies have implicated residues located in the D2 domain, including residues W87, W113, V115, K117, V118, Y120, Y121, K122, D123, K128, Y129, W130, Y131, E132, Y149, G153, V155, W156, D159, Y160 and E161 (refs 12, 29–33). Not all of these make direct contacts (within 4 Å) with the IgE-Fc. Of the residues identified by mutagenesis, eight interact directly with the IgE (W87, W113, K117, Y129, W130, Y131, E132 and W156), twelve are within three residues (V115, V118, Y120, Y121, K122, D123, K128, G153, V155, D159, Y160 and E161), and the remaining one (Y149) is buried in the hydrophobic core of D2.

The identification of the FcεRIα-binding site in IgE-Fc has implicated many regions in the Ce3 domain, including the Ce2–Ce3 linker, AB helix, CD loop, DE loop, EF helix, FG loop and G strand^{3,13,15,16,34}. Mutagenesis studies have identified residues P333, R334, F349, R376, S378, K380, R393, D409, E414, R427 and M430

as possible contact residues in the IgE-Fc. Of these residues, three are observed as contact residues (P333, R334 and R427) and one (M430) is within three residues of a contact. Six are distant from the IgE-Fc–FcεRIα interface (F349, R376, S378, K380, D409 and E414). Some mutations of these distant residues have little effect on FcεRIα binding (such as R376A, R376K, D409A, D409E and D409N), whereas others reduce binding to the receptor (such as R376E and D409R). Mutations distant from the FcεRIα binding site (in the AB helix, CD loop and EF helix) may indirectly affect receptor binding by altering the Ce3 conformation or the interdomain angle between Ce3 and Ce4.

The basis for IgE specificity and its implications

Diagrams of the amino-acid residues that lie within 4 Å of each other in site 1 and in site 2 are shown in Fig. 4a and b. Connecting lines highlight residues forming atomic contacts across the respective interfaces. Also shown are the residues that are found in the related human IgG receptors and in four subtypes of human IgG antibodies.

In site 1 there is little conservation of the residues that form the IgE-Fc–FcεRIα interface. Three residues are completely conserved (G335, D362 and N394 of IgE) in the Fc sequences, but there is poor conservation in the receptor sequences, except for the partial conservation of K117 and the relatively conserved Y129 (either tyrosine or phenylalanine). The conservation of K117 in three of the four receptors matches the complete conservation of D362 and G335 in the Fc sequences, potentially preserving one of the two salt bridges and one of the hydrogen bonds of site 1. For FcεRIα Y129, only tyrosine or phenylalanine is found, suggesting that this interaction may be conserved in IgG-Fc complexes with the FcγRs. However, Y131 of FcεRIα, which forms a large number of atomic contacts across the interface and is buried in a shallow surface

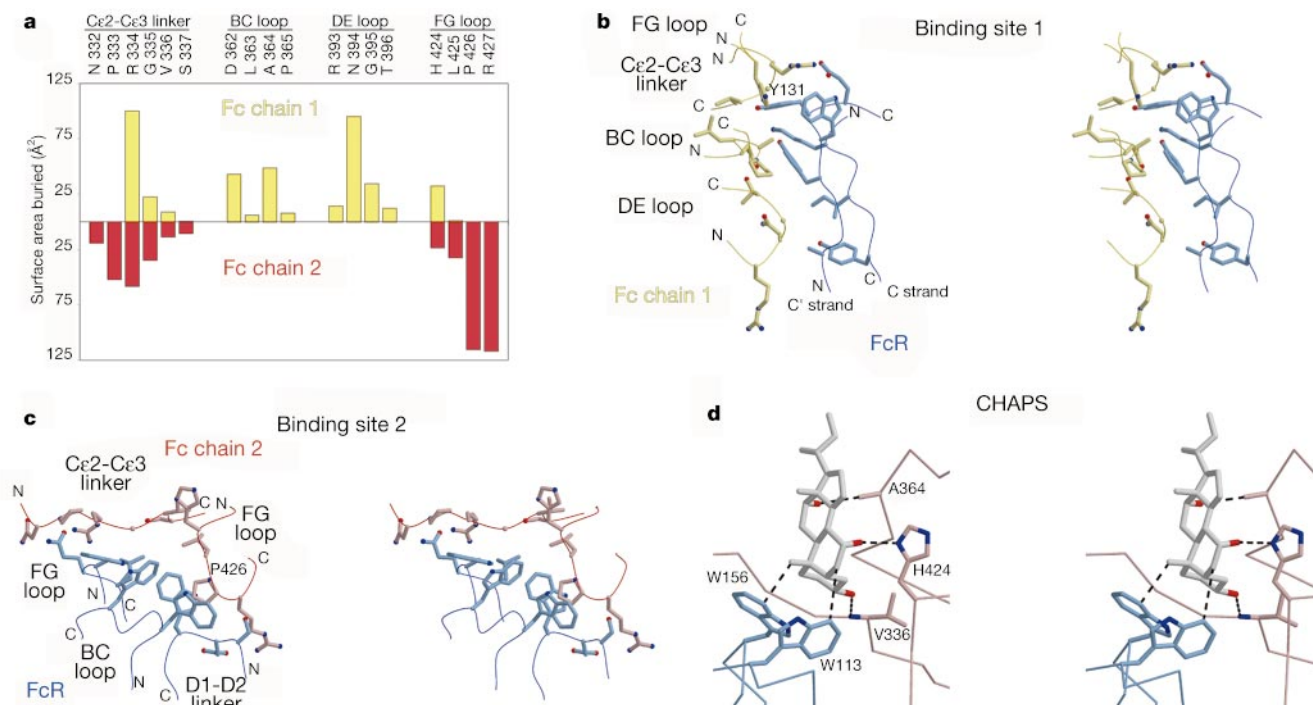


Figure 3 Details of the interactions in the IgE-Fc–FcεRIα complex at site 1 and site 2. **a**, Plot of the buried surface area of residues in the Ce3 domains of IgE-Fc. Residues buried in the site 1 interaction (top half; yellow bars) and residues buried in the site 2 interaction (bottom half; red bars) are shown. The IgE loops are identified above. Attached carbohydrate contributes 50 Å² of the buried surface area of N394. **b**, Stereo view of residue interactions at site 1. Binding loops are labelled at their termini, side chains of residues buried in the complex are shown, and Y131 is labelled. IgE-Fc and FcεRIα chains are yellow and blue, respectively. **c**, Stereo view of residue interactions at site 2.

Side chains of residues buried in the complex are shown. IgE-Fc and FcεRIα chains are red and blue, respectively. P426 (labelled) lies between the side chains of W87 and W110. **d**, Stereo view showing the binding of a CHAPS detergent molecule in the complex. Nitrogens are blue, oxygens are red, Fc carbons are pink, FcεRIα carbons are cyan and detergent carbons are white. Atoms less than 4 Å apart have dotted lines between them, and the residues are labelled. No density appears for the flexible end of the detergent, and those atoms are not modelled.

pocket on the IgE-Fc, is not conserved in the FcγRs (changing to either histidine or arginine). Given the central location of Y131 in the site 1 interface (Fig. 2d–f), this residue may generally be important in immunoglobulin specificity. Four of the five residues that contact Y131 in IgE change in the IgG-Fc sequences. Two allelic forms of FcγRIIa differ in only two residues (27 and 131) and in their binding to IgG2 (ref. 35). Residue 27 is not involved in the IgE-Fc–FcεRIα interface, but the change from R131 to H131 in site 1 may explain the loss of IgG2 binding for the R131 allotype. Residues in the four IgG subtypes are generally highly conserved in the site 1 interface (7 out of 9 are identical) compared with the significant variation in the FcγR residues.

Figure 4b shows the conservation of interactions in the site 2 interface. In the antibody sequences, P426 and L425 are absolutely conserved and P426 interacts with two absolutely conserved tryptophans in FcεRIα (W87 and W110), which form a hydrophobic pocket (Figs 2d, e and 3c). Site 2 also includes three residues (residues 332–334 of IgE) that in IgG affect binding to FcγRI. IgG1 binds with high affinity to FcγRI, whereas IgG2 does not. The binding affinity of IgG2 can be introduced into IgG1 by the substitution of residues LLG to PVA (in black in Fig. 4b), and IgG1 binding affinity is introduced into IgG2 by the reciprocal substitution^{36,37}. These residues in the IgE-Fc (residues 332–334) interact with the FG loop of FcεRIα (Figs 3c and 4b). Mutagenesis experiments have also shown that the transfer of the FG loop of FcεRIα to FcγRII confers detectable IgE binding³⁸. For site 2, the conservation of five residues and the additional functional data suggest a conserved binding mode across these members of the FcR

family. Variation in the FcγR FG loop sequences that contact the N-terminal linker region of the Fc fragment may provide key interactions that determine the specificity of FcγR–IgG pairs.

Discussion

The identification of two distinct binding sites in the IgE-Fc–FcεRIα complex and observed flexibility in Fc structures²⁷ (B. A. W. *et al.*, manuscript in preparation) suggest a kinetic scheme for IgE binding (Fig. 5a). Interdomain flexibility in the Cε3–Cε4 elbow could allow each Cε3 domain to transiently dissociate in the complex, leading to two pathways for the full dissociation of the IgE. Surface plasmon resonance studies of IgE-Fc–FcεRIα complexes show biphasic dissociation rates consistent with two distinct binding interactions^{16,34}. In this kinetic scheme, inhibitors specific for either site 1 or site 2 might accelerate the dissociation of receptor-bound IgE by capitalizing on the transient exposure of each site in the complex. Such inhibitors might prove useful in the treatment of acute allergic reactions in which dissociation of IgE from mast cells would be beneficial.

IgG-Fc and Fcγ receptors are structurally similar to the IgE-Fc and FcεRIα in the complex, and potentially could form analogous interactions. Superposition of a Cγ2 domain of IgG on the homologous Cε3 domain of IgE involved in binding site 2 places the second Cγ2 domain of IgG in close proximity to binding site 1 (Fig. 5b). Members of the Fc-receptor family have structural similarity, as shown by the superposition of the low-affinity IgG receptor FcγRIIb²² onto FcεRIα (Fig. 5b). Binding site 2 involves hydrophobic residues conserved in IgG and IgE antibodies and receptors, whereas binding site 1 and the receptor FG loop generate specificity through amino-acid variability. Compared with IgE, the binding of IgG to its receptors is more sensitive to the presence of N-linked carbohydrate in the Fc³⁹.

A model for an intact IgE antibody binding to FcεRIα is shown in Fig. 5c. In IgE, the Fc contains an additional pair of domains (Cε2) that separate the receptor-binding and Fab regions of the antibody. Calorimetric and binding studies show that the presence or absence

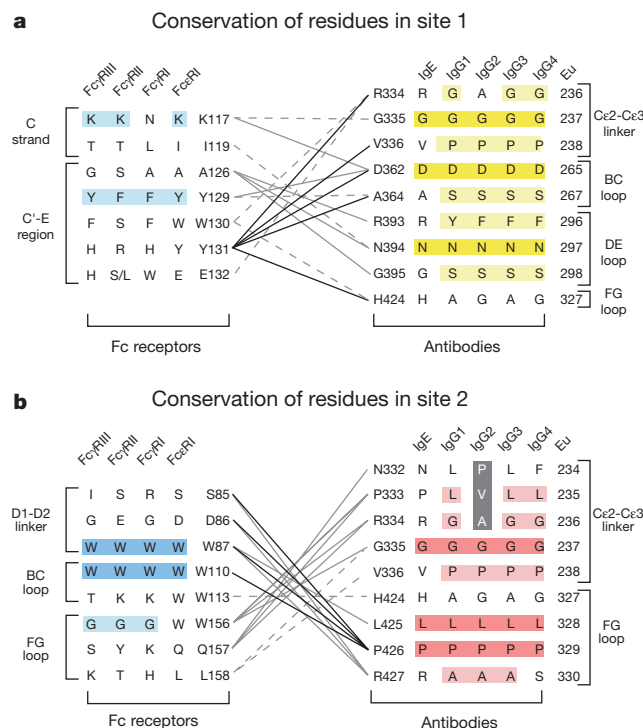


Figure 4 Conservation of amino-acid residues and contacts at the IgE-Fc–FcεRIα interfaces in IgG receptors and antibodies. Contacting residues are defined as interatomic distances less than 4 Å. **a**, Residues that interact in site 1, and their conservation in related human receptors and antibodies. Absolutely conserved residues are highlighted in bold, and partially conserved residues are lightly highlighted (yellow for IgE, blue for FcεRIα). Dark lines are drawn for residues making the largest number of contacts across the interface, lighter lines for intermediate numbers of contacts, and dashed lines for the fewest contacts. Numbering for the Eu protein (human myeloma IgG1) sequence is shown to the right. **b**, Residues that interact in site 2, and their conservation in related human Fc receptors and antibodies. Receptor residues are highlighted in blue, antibody residues in red. Three residues in IgG2 (P, V, A) that affect binding to FcγRI are boxed in black.

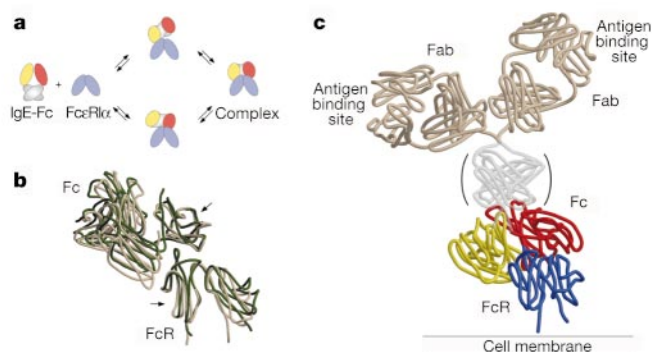


Figure 5 Implications of the complex. **a**, Kinetic scheme for the binding of IgE to its receptor. The interaction of each Cε3 domain with distinct surfaces of the FcεRIα structure suggests a kinetic scheme in which transient release of one of the Cε3 domains may occur within the complex. This could lead to two distinct pathways for the association and dissociation of the complex, consistent with the experimental observation of two distinct kinetic off-rates. Transient opening of the complex may allow inhibitors to enhance the dissociation of receptor-bound IgE by preventing the rebinding of an exposed Cε3 domain within the complex. **b**, Superposition of the Fc portion of an intact IgG antibody (1IGY)²⁷ and IgG Fc receptor FcγRIIb²² onto the IgE-Fc–FcεRIα complex. The IgE complex is shown in beige, the IgG homologues in green. Domains used to superimpose the structures are indicated with arrows. Only a minor adjustment of the other IgG domains is required to fit the IgE complex. **c**, A hypothetical model for an intact IgE-Fc–receptor complex. The IgE-Fc–FcεRIα complex is coloured as in Fig. 1, and antibody Fab regions are beige. The Cε2 domains of IgE are represented as paired immunoglobulin domains (transparent) inserted into the hinge regions between Fab and Fc, and shown in parentheses because no structural information is available for these domains.

of the Ce2 domains has no effect on the thermodynamic parameters associated with FcεRIα binding⁴⁰. In the absence of receptor binding, the Ce2 domains are probably tethered by flexible polypeptide segments, with no influence on the Ce3–Ce4 domain structure of IgE–Fc or on direct interactions with FcεRIα. In this model, Ce2 domains extend away from the receptor surface in the same direction as the Ce2–Ce3 linkers in the crystal structure, acting as an additional spacer between the Fabs and the Fc–FcR interface. Receptor binding at the Ce2–Ce3 linker promotes an overall bend to the IgE, consistent with previous fluorescence energy transfer studies⁴¹. Antibody bending would place the Fab regions away from the membrane (Fig. 5c), in position to bind multivalent antigens.

In contrast to receptors that dimerize and signal after binding a well defined ligand, Fc receptors act as adaptors between highly variable antigen-binding sites and the intracellular signal transduction machinery. Flexible linkers between the antibody Fab and Fc arms seem necessary to accommodate the variable structures of Fab–antigen complexes and the need to crosslink two or more Fc receptors to initiate signalling^{1,2,7}. Thus, FcR signal-inducing ligands (antigens) can promote co-localization of receptors but cannot form specific contacts that dimerize FcRs, as observed for hormone or cytokine receptor complexes. It remains unclear whether co-localization leads to a specific signalling structure or simply membrane relocation of the complex². Given the heavily glycosylated state of the FcεRIα chain, it is unlikely that the D1–D2 domains could form protein–protein contacts to initiate signalling. Contacts between neighbouring antibodies, α-chain transmembrane segments, or FcεRI β- or γ-subunits, however, might mediate the assembly of a distinct signalling complex after antigen-induced multimerization.

The crystal structure of the IgE–Fc–FcεRIα complex clarifies the atomic interactions that regulate the specificity and stoichiometry of protein–protein interactions underlying allergic reactions. The observation of bound CHAPS molecules provides a lead for the structure-based development of novel inhibitors for the treatment of allergy, anaphylaxis and asthma. Insights into antibody binding to Fc receptors may also provide new approaches to treating autoimmune disorders and to the improvement of cancer therapies based on antibodies. □

Methods

Crystallization of the human IgE–Fc–FcεRIα complex

We expressed the Ce3–Ce4 domains of human IgE–Fc (B. A. W. *et al.* manuscript in preparation) and FcεRIα¹² in insect cells. The purified IgE–Fc protein includes four non-wild-type residues generated by the construct (ADPC), followed by wild-type amino acids 330–547 (ref. 42). The cysteine (construct residue 329) is displaced by one amino acid from the wild-type IgE–Fc C328, and forms an interchain disulphide bond in about 95% of the isolated IgE–Fc. Complexes of the Ce3–Ce4 domains of IgE and wild-type FcεRIα produced poorly diffracting crystals (Table 1, crystal form I). As the receptor is heavily glycosylated (~33% carbohydrate by weight), and the carbohydrate sites are dispersed on the receptor surface, we selectively removed a subset of these attachment sites to improve the protein crystallization. We expressed a carbohydrate mutant of the receptor lacking three of the seven wild-type carbohydrate sites (74, 135 and 140)¹⁸ in insect cells. The receptor triple mutant FcεRIαΔ4–6 was purified by affinity chromatography¹².

We incubated purified IgE–Fc and FcεRIαΔ4–6 or FcεRIα to form a complex, and purified it by gel-filtration chromatography using a Superdex 75 column (Pharmacia). The complex was concentrated to 10 mg ml⁻¹, and hanging drop crystallizations carried out at 23 °C. Form I crystals of the wild-type IgE–Fc–wild-type FcεRIα complex grew from 1.4 to 1.6 M ammonium sulphate and 100 mM Tris pH 8.5, over a period of 8–12 months. Form II crystals grew from purified wild-type IgE–Fc–FcεRIαΔ4–6 complex using 100 mM Tris, pH 8.5, 1.4–1.6 M ammonium sulphate and 8 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulphonate (CHAPS) in several days. Neither crystal form grew longer than about 110 μm. Crystals were transferred to buffer (form I, 2.1–2.7 M ammonium sulphate and 100 mM Tris pH 8.5; or form II, 1.6–2.0 M ammonium sulphate, 100 mM Tris pH 8.5 and 0.8 mM CHAPS) and frozen in the same buffer supplemented with 15% glycerol. Data sets were collected at ALS 5.0.2 beamline and the APS DND-CAT 5-ID-B beamline at ~160 °C using an ADSC Quantum 4 detector or a MarCCD detector. Significant crystal decay was observed during data collection. Images were processed using the HKL suite of programs⁴³, and intensities were adjusted using TRUNCATE⁴⁴. Form I crystals belong to space group P4₁2₁2 with *a* = *b* = 126 Å, *c* = 129 Å, and form II crystals belong to the space group R32 with *a* = *b* = 192.8 Å and *c* = 302.4 Å

(hexagonal setting). Both crystal forms contain one IgE–Fc–FcεRIα complex in the asymmetric unit.

Crystal structure determination and refinement

Using the AMoRe⁴⁴ and EPMR⁴⁵ programs, we carried out molecular replacement on the form II low-resolution data with model coordinates from the 2.4 Å structure of the receptor¹². Normalized structure factors were critical to the success of the search in AMoRe. Both AMoRe and EPMR produced crystallographically equivalent locations for the receptor. 2F_o–F_c electron density maps with phases from the receptor revealed density corresponding to the two Ce3 domains of the Fc. Models for the core residues of Ce3 and Ce4 were created (B. A. W. *et al.*, manuscript in preparation) based upon homologous residues from IgG Mab231 (ref. 46). A new 2F_o–F_c map using phases from the receptor and core residues of Ce3 showed density for the two Ce4 domains. A model for the core residues in Ce4 was inserted. Rigid body refinement of the receptor, the core residues in Ce3, and the core residues in Ce4 produced an *R*_{free} of 45%. 2F_o–F_c maps and composite omit maps revealed electron density for protein and carbohydrate atoms absent from the model.

Refinement was continued with the form II data using all data with $|F| > 0$ up to the resolution shell where the $\langle I/\sigma \rangle$ dropped to 1.5 (3.25 Å). The data beyond 3.5 Å are weak, and we report the 3.5 Å structure and statistics here. Model building was done with the program O⁴⁷ and refinement carried out with CNS⁴⁸. The *R*_{free} set comprises 5% of the data that were selected using thin resolution shells. Strong non-crystallographic symmetry restraints (300 kcal mol⁻¹ Å⁻² on positions and 2 Å² on *B*-factors) were separately imposed on the Ce3 and Ce4 domains, while excluding the receptor-binding loops and the Ce3 carbohydrate. The ratio of the number of reflections to the number of atoms in the refinement is relatively high for this resolution (~5:1). A bulk solvent correction was applied and individual *B*-factors were used, as they lowered the *R*_{free} from 29.3% to 27.3% relative to grouped *B*-factors. The high overall *B*-factors are consistent with the weak diffraction from these crystals, but good electron density is observed throughout the model. After inserting all missing loops from the protein, five CHAPS molecules were located as large peaks of positive density in F_o–F_c maps. Of the 620 amino acids in the complex, 14 are missing from the model, all at chain termini: FcεRIα residues 174–176; Fc chain 1 residues 326, 327 and 545–547; and Fc chain 2 residues 326–328 and 545–547. Current refinement statistics are summarized in Table 1. Figures were made using the programs Molscript⁴⁹ and Grasp⁵⁰.

The form I crystal structure was solved by molecular replacement with AMoRe using the form II model of the complex. A single solution had a correlation coefficient of 26.0 with the next highest peak 13.5 (using normalized *F*s). Searches in the enantiomorphic space group gave correlations lower than 12.3. Given the low resolution of form I crystals, refinement was limited to rigid body minimization.

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